# CAPTIVE-REARING OF *SPHAEROTHECA BREVICEPS* FROM EARLY EMBRYONIC STAGES TO OVER THREE-YEAR-OLD ADULT[1]

MRINALINI VIRKAR[2,3], SAVITA JOSHI[2,4] AND S.L. SHINDE[2,5]

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[2]Zoology Group, Animal Sciences Division, Agharkar Research Institute, G.G. Agarkar Road, Pune 411 004, Maharashtra, India.
[5]Email: slslab@hotmail.com

*Sphaerotheca breviceps* is a burrowing frog of Family Ranidae, widespread in peninsular India. 40 eggs of this species were collected from the wild, and reared successfully under laboratory conditions using five different set-ups, depending upon their developmental or seasonal needs. Rapid development, juvenile cannibalism, seasonal behaviour of aestivation, and possibility of breeding under laboratory conditions are the highlights of rearing this species. The development including metamorphosis was completed in 35 days. The adult males showed vocal sacs and nuptial pads, and gave mating calls during three successive breeding seasons. The adult survivors showed an average life span of three years, one of which survived for 1289 days. Their rapid development and overall health during the course of the study indicate that the species can successfully adapt to variations in temperature and humidity in the laboratory.

**Key words**: *Sphaerotheca breviceps*, frog, laboratory rearing, development, juvenile cannibalism, aestivation, mating calls, life span

## INTRODUCTION

The Indian Burrowing Frog *Sphaerotheca breviceps* (Schneider 1799) is distributed in India, Sri Lanka and Myanmar (Boulenger 1890, 1920). We have identified three locations on the Pune-Alandi and Alandi-Chakan Road that have a dense population of frogs (Fig. 1a). During the monsoon season of 1997, we came across an unusual spawn similar to that of *Microhyla ornata*. Intrigued, we collected forty eggs from this spawn that had about three hundred eggs, and reared them under laboratory conditions. During the course of the study, S.K. Dutta identified the species as *Sphaerotheca breviceps* from the two eventually grown adults. Hitherto known information on the habits of this species is little; more information on its ecology and behaviour was, therefore, felt necessary (Daniel 1975, 2002). In this paper, we report our observations of 1289 days on rapid development, voracious feeding, good acclimatization to laboratory conditions, cannibalism on metamorphs and distinctive mating calls of the Indian Burrowing Frog.

## STUDY AREA

We have been visiting three ponds along the Pune – Chakon road to observe their rich amphibian fauna. Three sites were identified and marked as Location 1, Location 2 and Location 3. Location 2 and 3 are adjacent to each other on Alandi – Chakan road, 27 km from Pune and 6 km from Alandi. Location 1 is situated at 18° 38' 22" N and 73° 52' 45" E on Pune – Alandi road, 16.6 km from Pune and 4.4 km from Alandi (Fig. 1a). It comprised of eleven temporary rainwater puddles and one big seasonal pond (Fig. 1b). The pond contains water for about nine months of the year (July-March) under conditions of average rainfall in monsoon. The amphibian species found at these sites are *Hoplobatrachus tigerinus*, *Microhyla ornata* and *Bufo melanostictus*. *M. ornata* is the dominant species found in Location 1. The spawn of *S. breviceps* eggs was noticed only once at Location 1 in puddle number 6 (Fig. 1a & b).

## MATERIAL AND METHODS

**Rearing of developmental stages**: During the early period of embryonic and tadpole stages, the eggs were kept in a plastic tub containing dechlorinated water. The tadpoles showed well-developed hind limbs at 23 days, corresponding to Gosner stage (CGS) 38 (Gosner 1960). About 5 ml of spinach extract was added every day and about 2 ml of plankton concentrate was added every alternate day to the water as feed. Planktons were collected from the wild every fortnight and maintained in the laboratory until fresh replenishments were obtained. The spinach debris or excreta was removed every day with a wide-mouth pasteur pipette, and the water in the tub was replenished every alternate day. The tadpoles developed forelimbs at 31 days (CGS 42). They were then transferred to a plastic bucket containing tap water, dechlorinated by storing for at least two days, and coarse sand. In the bucket, coarse sand was arranged on one side in a slope with height 2 cm above the water level. The tadpoles were kept under this set up till they metamorphosed into froglets around the 35th day (CGS 46).

**Rearing of newly metamorphosed froglets**: When metamorphosis was complete, the young froglets were transferred to an aquarium with steps of thermocol as shown

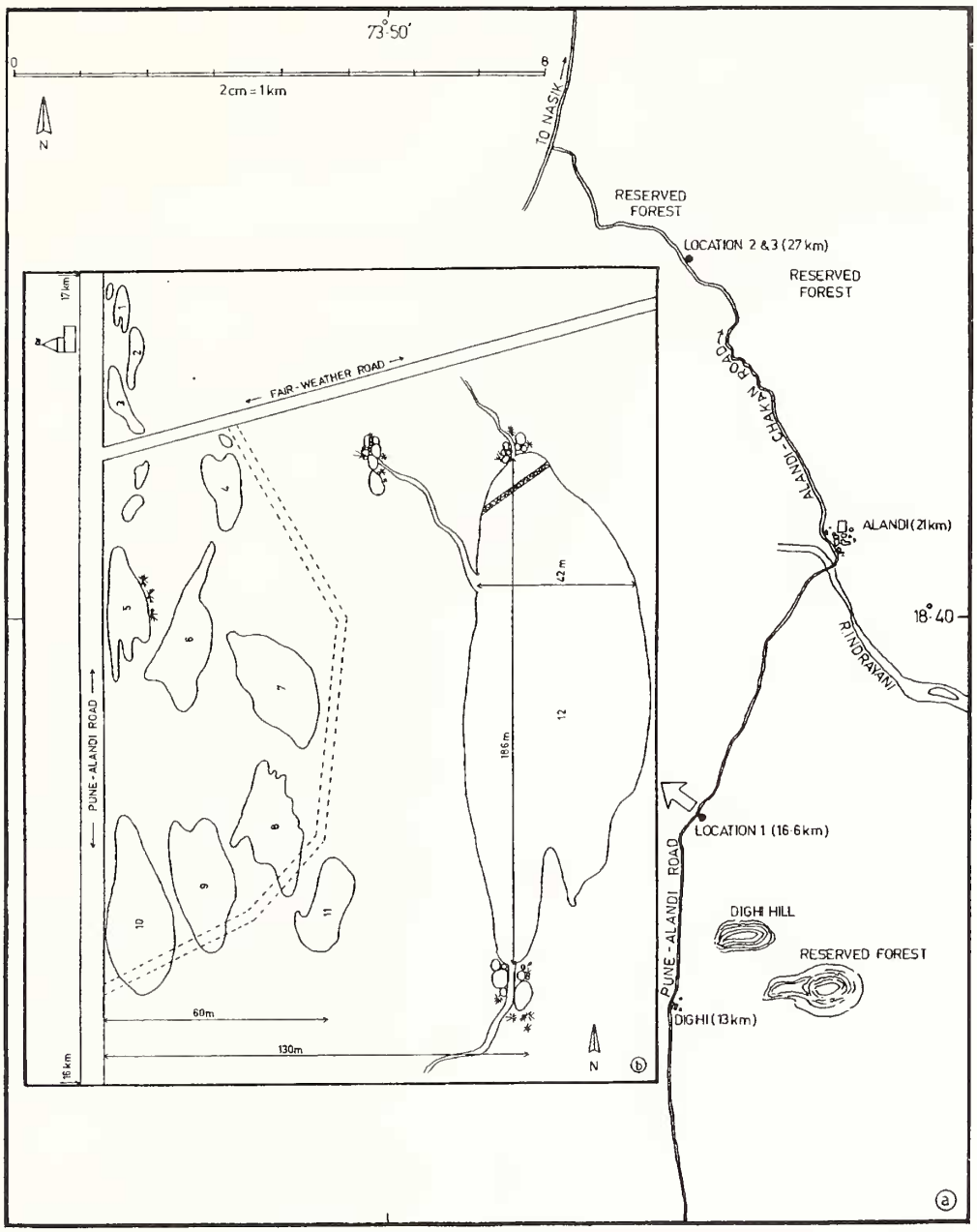


Fig. 1: (a) Location map of dense population sites of frogs on Pune-Alandi Road and Alandi-Chakan Road; (b) Schematic site map of the puddles and pond on Pune-Alandi Road where spawn of *Sphaerotheca breviceps* was found

in Fig. 2a. The thermocol steps were covered with filter papers that were changed once a week or when found soiled. A few pebbles were kept on the steps. Water was added up to the lowermost thermocol step and was changed every alternate day by tube siphoning. Trials were made to feed the froglets black ants, de-winged or vestigial winged *Drosophila* flies, termites, red cotton bugs, besides successfully feeding them commonly available tubificid worms of genus *Limnodrilus*. Black ants and termites were collected from nature. *Drosophila* flies and red cotton bug (*Dysdercus koenigii*) nymphs were from cultures maintained in the laboratory.

**Rearing of young and mature adults**: The aestivation set up was prepared based on initial observations at the onset of the first winter and summer respectively, during rearing of the froglets. Two aluminium trays were kept on either ends of an aquarium. One tray was filled with non-sticky garden soil while the other was half-filled with water. A thermocol sheet covered with a sheet of filter paper, which was changed routinely, was placed between the two trays (Fig. 2b). A petri plate containing weighed quantity of tubificid worms was kept on the covered sheet of thermocol every evening. The soil in the tray was kept moist by sprinkling water over it as

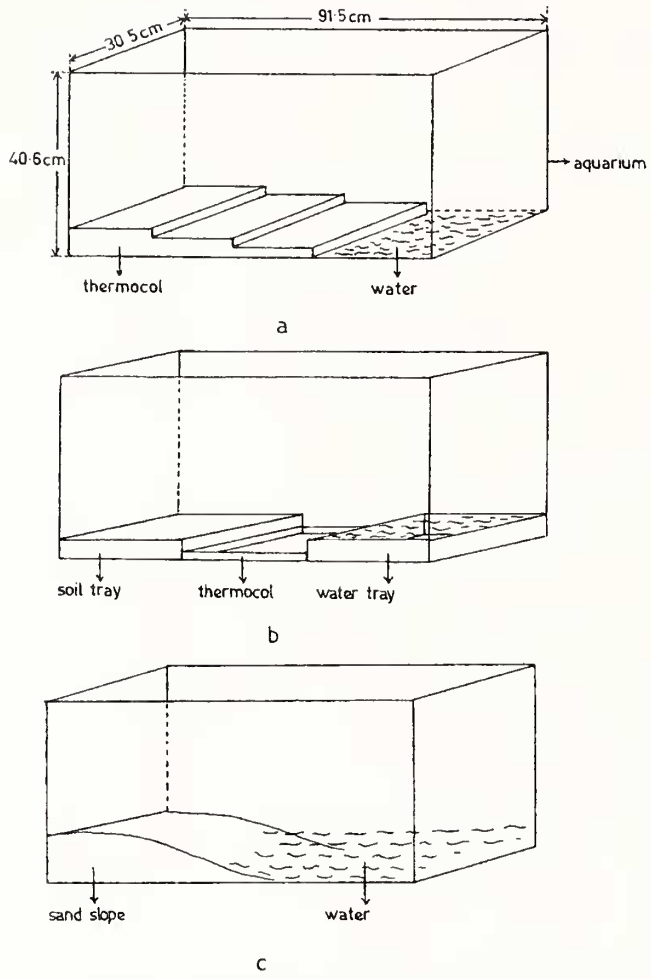


Fig. 2: Different laboratory habitats used for rearing *S. breviceps*; (a) Habitat used for newly metamorphosed froglets; (b) Habitat used for young and adults for aestivation; (c) Habitat used during monsoon for adults

and when required. The soil from the tray was changed every 3 months. The water tray was removed, cleaned and refilled every day with dechlorinated tap water maintained in the laboratory. During each monsoon period, between July and October, all the frogs were transferred into another aquarium set up. This contained a 15 cm high slope of sand, starting on one side and terminating in the middle of the aquarium. The other half of the aquarium was filled with 10 cm water with few small stones close to the sand bed (Fig. 2c).

**Laboratory temperature and humidity**: The eggs, tadpoles and adults were maintained in the laboratory under normal uncontrolled conditions of temperature and humidity. The temperature in the laboratory between March and June was 25 °C to 40 °C, between July and October was 20 °C to 35 °C and between November and February was 10 °C to 30 °C. The relative humidity in the laboratory during summer was 40% to 60%, during monsoon 60% to 90% and during winter 50% to 70%.

## RESULTS AND DISCUSSION

**Spawn and eggs**: During a routine survey, on July 27, 1997, a spawn of *S. breviceps* was collected from puddle no. 6 (Fig. 1b) at Location 1. It was a round spread of jelly holding eggs. Eggs were reddish mustard in colour. The spawn was of moderate size consisting of about 300 eggs. The appearance of the spawn and eggs was different from the spawns and eggs of other frogs and toads in that area. So, we carefully separated a small piece of the spawn at 1030 hrs. The eggs were well formed and in the dorsal lip stage (CGS 10). The eggs were counted in the laboratory and found to be 40 in number. We observed the eggs under a binocular zoom microscope, and found them to be in the early neurula stage, at approximately 9 hrs of age (CGS 13).

**Developmental span and feeding habits**: The present report of developmental span from eggs to metamorphosed froglets in *S. breviceps* is 35 days, which is shorter by 10 days in comparison with an earlier report (Mohanty-Hejmadi *et al.* 1979). *S. breviceps* tadpoles and adults accepted all kinds of food supplied. They readily and greedily swallowed tubificid worms throughout their life span under laboratory conditions. There is a drastic shift in the feeding habit of *S. breviceps* from herbivorous tadpoles to wormivorous froglets and frogs, when reared under laboratory conditions. These observations conform to inferred natural food habits recorded as herbivore in tadpoles (Sekar 1992) and insectivorous in frogs of this species (Mohanty-Hejmadi and Acharya 1982). The overall growth rate of the species appeared to be considerably rapid and they remained healthy during their life span under laboratory conditions. These observations led us to believe that the species could serve as a good amphibian laboratory model.

**Juvenile cannibalism**: All forty individuals of *S. breviceps* observed during this study were reared together. Soon after completion of their metamorphosis we were trying to feed young froglets with various kinds of food as mentioned earlier, when we observed four froglets being swallowed by their fellow frogs during this early phase after metamorphosis. We not only observed the process of swallowing, but also noticed the bulging bellies of the cannibalistic individuals. We increased the tubificid quota substantially, which they relished, to avoid such instances. No more act of cannibalism was observed throughout the remaining course of rearing after this change. The intraspecific predation among amphibians is well known. Juvenile cannibalism is reported earlier in *R. pipiens* and *R. temporaria* as reviewed by Polis and Myers (1985). This is the first report on juvenile cannibalism in *S. breviceps.*

**Aestivation**: During the course of the study, the young adults underwent aestivation during which they were seen

hiding below the filter paper or burrowing under the thermocol sheet. It was fascinating to see most of them hiding under the filter paper cover. We, therefore, prepared a special set up for burrowing activity (Fig. 2b). The young adults showed enhanced feeding prior to aestivation as was evident from the average amount of food consumed (about 10 gm/frog/day); from November, the extent of feeding reduced drastically (about 2.5 gm/frog/day). As the room temperature at night dropped below 20 °C, the frogs buried themselves in the moist soil kept in one of the trays. They used their forelimbs as shovels to remove the soil sideways while burying into the soil. The dug out soil was cleared quickly with the help of hind limbs. It is inappropriate to say that the structure of hind limbs enables this frog to burrow as reported previously (Boulenger 1890). Some of them used to emerge out of these hideouts during late evenings for meagre feeding (less than 2 gm/frog/day) before burrowing into the soil again. The feeding almost ceased during December and January. The frogs looked thinner and pale after aestivation. During summer, the frogs showed burrowing behaviour only during daytime. They continued feeding (less than 3 gm/frog/day) until the onset of the rainy season in June. The surviving individuals showed similar behavioural patterns in subsequent years too. As adults grew, the average amount of food consumed increased during subsequent years (about 15 gm/frog/day), but the extent of meagre feeding during aestivation remained unchanged (about 3 gm/frog/day). We observed such behaviour for seven complete cycles. This behaviour in young adults indicates that seasonal cyclical changes are built intrinsically.

**Mating calls**: This was another important feature of *S. breviceps* observed in one-year old adults, around the onset of monsoon. It was striking that the eleven individuals in the laboratory were responding to the natural seasonal changes outside. This indicates built-in physiological rhythms related to breeding, which may not necessarily depend upon environmental cues. At the beginning of breeding season in 1998, the surviving frogs showed development of vocal sacs. The call notes were short syllables "*auan*" expressed in quick succession. Each call group was composed of a series of syllables. The frequency and pitch of the calls gradually increased towards the end of each call group. As previously described, the call notes were neither the short syllables "*Rut-Rut-Rut*" (Rao 1915) nor the soft "*awang*" (Daniel 1975, 2002), but each call group is composed of a large number of calls (Kanmadi *et al.* 1994) that can be heard from a distance of about 15 m. On observing this, we developed a special set up in the hope of possible breeding (Fig. 2c). The entire lab was filled with loud, but not shrill mating calls during late evenings, prompting us to rush to the laboratory every morning for two weeks; we expected to see some eggs, but this never happened, because all the adults reared were males. This prompted us to carefully check vocal sacs and nuptial pads on forelimbs of all the individuals. Since all of them had vocal sacs as well as nuptial pads, egg laying was no more a possibility. The mating calls were heard from the surviving adults during two more breeding seasons that followed.

**Life span**: The objective of these studies was to learn the total life span of this species. In terms of surviving numbers, it is obvious that the individuals were more protected under laboratory conditions than in their natural surroundings. In terms of other natural factors, including possible variety in feeding, they may have been deprived of several unknown factors. The healthy condition of the surviving adults, throughout the course of this study, indicates that the observed life span in their natural habitat may not be substantially different. Our observations indicate that majority of the adult individuals survived an average life of three years. The last individual survived for 1289 days. This may serve as an indicator of average life span of this species in the nature too. This is first report on the longevity of *S. breviceps* under laboratory conditions or otherwise.

**Life profile under laboratory conditions**: Our initial strategy of fixing froglets at 5 mm snout-vent length increment retrospectively appears to be an unwanted intervention. This could have prevented the 'all-male-situation' of surviving adults. Success of rearing this species during the initial year prompted us to search for more spawn(s). In spite of our best efforts, we could not locate one more spawn at the localities identified by us (Fig. 1a & b) between 1998 and 2003. The species may have become extinct from the study localities. The present study opens up a possibility of rehabilitation of this species by rearing them under laboratory conditions.

The overall trend of life of this frog under laboratory conditions highlights other important features of this species. The foremost being that they remain healthy in captivity if their basic needs are provided in time. Although voracious, their feeding behaviour is flexible and it is surprising that they adjusted well to feeding on tubificid worms. Barring juvenile cannibalism, they co-existed well in captivity sharing their resources. Rapid development, juvenile cannibalism, cyclical behaviour of aestivation and strong possibility of breeding under laboratory conditions were the highlights of rearing this species. The entire chronological sequence of events of rearing *S. breviceps* individuals is tabulated in Table 1 to help subsequent work on amphibian studies, to keep track of this long-lasting exercise and also to keep a record of available specimens for study.

**Table 1**: Chronological events of rearing the Indian Burrowing Frog *Sphaerotheca breviceps* from developmental stages to over three-year-old adult

| Duration | Highlights of the Event | Number of survivors |
|---|---|---|
| July 29, 1997 | Observed a floating spread of spawn with reddish mustard coloured eggs; the spawn was medium-sized containing about 300 eggs out of which 40 eggs were collected at dorsal-lip stage (CGS 10) and brought to the laboratory at neurulation stage (CGS 13). | 40 |
| Up to August 28, 1997 | Two tadpoles died (CGS 42) during this 30 day period: one died on 27th August (SVL = 33 mm) and another died on 28th August (SVL = 35 mm). | 38 |
| August 30-September 5, 1997 | Metamorphosis completed (CGS 46) in 35 ±3 days | 38 |
| September 1-2, 1997 | Three froglets were fixed in Bouin's fixative (SVL = 15 mm) and preserved in 70% ethanol. | 35 |
| September 1-13, 1997 | Four froglets were swallowed by their fellows. | 31 |
| September 14, 1997 to June 30, 1998 | Twenty froglets/young adults (measuring 15-45 mm SVL during the course) were fixed in Bouin's fixative and preserved in 70% ethanol or formalin fixative after a 5 mm increase in their SVL; of these two specimens were handed over for species identification.<br><br>During the onset of the first cycle of aestivation, froglets scratched and dug into thermocol sheets or hid themselves under filter paper. No significant change in behaviour was noted during the consequent cycles. | 11 |
| July 1 to September 25, 1998 | An aquarium, with a slope of coarse sand at one end and water at the other end was prepared and the frogs were transferred into this aquarium. Mating behaviour in the form of loud mating calls was noted. | 11 |
| August 13, 1998 | Soil tray - thermocol sheet - water tray set-up was prepared for the forthcoming aestivation cycle. | 11 |
| September 26 to December 23, 1998 | Three frogs died naturally, two of which were fixed in formalin and one was discarded. | 8 |
| November 1998 to June 1999 | Second cycle of aestivation was observed;<br>One adult died (preserved). | 7 |
| July to October 1999 | Second breeding season when behaviour of mating calls was noted. | 7 |
| November 1999 to June 2000 | Third cycle of aestivation was observed<br>Five adults died (preserved). | 2 |
| July to October 2000 | Third breeding season when mating calls were heard.<br>One adult died (preserved). | 1 |
| November 2000 to February 2001 | Fourth cycle of aestivation. | 1 |
| March 9, 2001 | Last adult died (preserved). | 0 |

CGS = corresponding Gosner Stage; SVL = snout-vent length

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